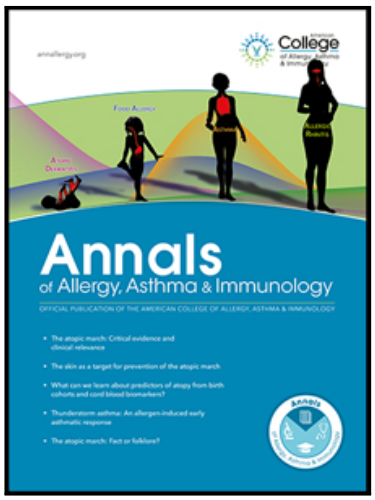

Small airway function in children with mild-to-moderate asthmatic symptoms and healthy controls

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Small airway function in children with mild-to-moderate asthmatic symptoms and healthy controls

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Abstract

Background: Clinical significance of small airway obstruction in mild pediatric asthma is unclear. Objective: To evaluate small airway properties in children with mild-tomoderate asthmatic symptoms, and the association of small airway function with asthma control and exercise-induced bronchoconstriction (EIB). Methods: Children (5-10 years) with either recurrent wheezing (n=42) or persistent troublesome cough (n=16), and healthy controls (n=19) performed impulse oscillometry (IOS), spirometry, and multiple-breath nitrogen washout (MBNW) test. Exhaled nitric oxide (NO) was measured at multiple flow rates to determine alveolar NO concentration (CALV). Asthma control was evaluated with the Childhood Asthma Control Test (C-ACT), short-acting beta2-agonist (SABA) use within the past month, and asthma exacerbations within the past year. Results: IOS, spirometry, and exhaled NO indices which are related to small airway function differed between children with recurrent wheezing and healthy controls, whereas only forced expiratory flow at 25-75% of the forced vital capacity (FEF25-75) was associated with persistent cough. MBNW indices showed no difference between the groups. Among symptomatic children, conducting airway ventilation inhomogeneity (Scond) and CALV were associated with asthma exacerbations (p=0.028 and p=0.002, respectively), and lung clearance index (LCI) and CALV

were associated with EIB (p=0.044 and p=0.004, respectively). None of the proposed small airway indices was associated with the C-ACT score or SABA use. Conclusion: Subtle changes were observed in the proposed small airway indices of IOS, spirometry, and exhaled NO among children with mild-tomoderate recurrent wheezing. Small airway dysfunction, expressed as ventilation inhomogeneity indices and CALV, was also associated with asthma exacerbations and EIB.

Keywords
pediatric asthma, early wheezing, small airways, lung function testing, impulse oscillometry, spirometry, multiple-breath washout, exhaled nitric oxide, asthma control, bronchial hyperresponsiveness

Abbreviations: AUC, area under receiver operating characteristic curve; AX, area under the reactance curve; C_{ALV}, alveolar nitric oxide concentration; CI, confidence interval; C-ACT, childhood asthma control test; EIB, exercise-induced bronchoconstriction; FEF_{25-75}, forced expiratory flow between 25% and 75% of the forced vital capacity; FE_{NO}, fractional exhaled nitric oxide; $FE_{NO}50$, FE_{NO} at 50 ml/s; FEV_1, forced expiratory volume in 1 second; FRC, functional residual capacity; FVC, forced vital capacity; IOS, impulse oscillometry; IQR, interquartile range; J_{NO}, bronchial nitric oxide flux; LCI, lung clearance index; MBNW, multiple-breath nitrogen washout; MBW, multiple-breath washout; NO, nitric oxide; r^2, goodness of fit; R5, respiratory resistance at 5 Hz; R20, respiratory resistance at 20 Hz; R5-20, difference between respiratory resistance at 5 and 20 Hz; ROC, receiver operating characteristics; SABA, short-acting $beta_2$-agonist; Sacin, ventilation inhomogeneity in the acinar structures; Scond, ventilation inhomogeneity in the conducting airways; X5, respiratory reactance at 5 Hz.

INTRODUCTION

Bronchial biopsies and post-mortem studies have demonstrated the involvement of small airway inflammation in asthma,[1,2] supported by clinical studies on the association between small airway dysfunction and clinical expression of asthma.[3,4] In children, abnormalities in the proposed small airway indices have been related to uncontrolled or severe asthma,[5-7] but their potential to distinguish milder disease changes remains debated.[8,9] It is plausible to hypothesize small airways to be involved to some extent in most patients with asthma,[10] even in children. Early involvement of

small airways has also been recognized as a risk factor for future chronic obstructive pulmonary disease.[11] Furthermore, with the emergence of small-particle aerosols that reach the lung periphery, accurate measures for small airways are necessitated to identify and follow patients who might benefit from this treatment. However, conventional lung function measures, such as forced expiratory volume in 1 second (FEV_1), mainly reflect large airways, and no gold standard measures for small airways exist. Potential methods for assessing small airway properties include impulse oscillometry (IOS), spirometry, multiple-breath washout (MBW) tests, and extended exhaled nitric oxide (NO) analysis.

IOS measures respiratory resistance and reactance by analyzing responses to pulse signals of different frequencies. High frequency resistance values, such as resistance at 20 Hz (R20), are considered to reflect large airway resistive properties, whereas lower frequencies, such as resistance at 5 Hz (R5), reflect the whole respiratory system.[12] Small airway obstruction is thought to result in frequency-dependence of resistance, and thus increased difference between R5 and R20 (R5-20).[5] Negative reactance values reflect the capacitative properties of the lung periphery, and thus reactance at 5 Hz (X5) and area under the reactance curve (AX) are considered to reflect small airway function.[5,12]

During forced expiration, small airway obstruction is postulated to contribute to airflow limitation during the mid- and end-phases of the forced vital capacity (FVC). This results in diminished mid-expiratory flow rates, without significant effects on FEV_1 which mainly reflects large airway function at the initial part of forced expiration.[13] Mid-expiratory flow rates can be evaluated by calculating the forced expiratory flow between 25% and 75% of the FVC (FEF_{25-75}), which is considered to reflect the medium- and small airway function.

MBW measures ventilation distribution homogeneity by analyzing the washout pattern of an inert marker gas. Lung clearance index (LCI) is postulated to reflect the overall ventilation

inhomogeneity secondary to small airway disease.[14] The washout pattern over several breaths aims to separate convection-dependent ventilation inhomogeneity in the small conducting airways (Scond) and diffusion-convection-dependent inhomogeneity in the acinar structures (Sacin).[14]

Airway inflammation enhances production of NO in the bronchial epithelium.[15] At low flow rates, such as 50 ml/s, the fractional exhaled NO (FE_{NO}) mainly reflects NO from the central airways.[16] With increasing flow rates, contribution of proximal airways decreases and FE_{NO} represents alveolar NO.[16] Applications with multiple exhalation flow rates have been created to separate alveolar NO concentration (C_{ALV}) from bronchial NO flux (J_{NO}).[15]

Our primary aim was to investigate whether small airway dysfunction is present in young children with mild-to-moderate asthmatic symptoms by using different indicators of small airway involvement: IOS, spirometry, multiple-breath nitrogen washout (MBNW) test, and extended FE_{NO} measurement. The secondary aim was to evaluate the association of these measurements with asthma control and exercise-induced bronchoconstriction (EIB). Finally, considering their proposed relationship with small airway function, we hypothesized that the small airway indices of the compared methods would be associated with each other.

METHODS

Subjects

The symptomatic children (n=58) aged 5-10 years were recruited from patients that were referred to a tertiary unit between April 2016 and May 2017 due to symptoms indicating probable asthma. Inclusion criteria were: 1) symptoms indicating probable asthma: ≥3 episodes of wheezing, or troublesome cough lasting continuously for ≥6 weeks without indications of alternative differential diagnoses, 2) no other chronic or acute diseases of the respiratory system, and 3) acceptable lung function measurements at the first visit. At the first visit, the children first performed IOS and then spirometry measurements, combined with an exercise challenge test. MBNW and extended FE_{NO} measurements were performed at the second visit within 14 days. The parents of 65 symptomatic children delivered an informed consent for study participation. Thereafter, 4 children were excluded due to symptoms of a respiratory tract infection before the second visit, 2 children because of failure to deliver the research questionnaires, and 1 child later refused blood sampling.

Nineteen healthy controls aged 5-10 years were recruited through an announcement which was targeted to the personnel of the hospital from which the patient were recruited. Inclusion criteria were: 1) no symptoms or diagnosis of acute or chronic respiratory disease, and 2) no systemic disease with possible direct or indirect respiratory effects. All of the healthy controls who filled the inclusion criteria performed successful IOS and spirometry measurements and were included in the study. Healthy children performed MBNW, extended FE_{NO}, IOS, and spirometry measurements in the aforementioned order during one visit.

All children were free from respiratory tract infections for at least 2 weeks, and possible asthma-control medications ceased at least 4 weeks prior to the lung function measurements according to national practice guidelines. This was done to minimize their possible confounding effect on lung function. Parents filled a questionnaire on the child's family history, health status, and symptoms.

Asthma control was evaluated with the Childhood Asthma Control Test (C-ACT) (a score of ≤19 indicated inadequate asthma control[17]), use of short-acting beta$_2$-agonist (SABA) within the past month, and asthma exacerbations (episodes of shortness of breath or wheezing) within the past year.

Impulse oscillometry

IOS (Masterscreen IOS, Carefusion, Hoechberg, Germany) measurements were performed as described earlier,[18] according to international recommendations.[19] During all lung function measurements, the children were in a sitting position and breathed through a mouthpiece using a nose clip. To minimize pressure losses in IOS, cheeks were supported by the investigator. The measurement was accepted when ≥3 regular tidal breathing patterns lasting ≥20 seconds without signs of artefacts, such as apnea, speaking, swallowing, or air leak were obtained. Table 1 summarizes the postulated small airway indices of the different measurements used in the study and their hypothetical physiological determinants.

Spirometry

Spirometry (Masterscreen Pneumo, Carefusion, Hoechberg, Germany) was performed according to international guidelines,[20] with ≥3 successful measurements recorded, and the highest values of FEV_1 and FVC used in the analyses. Flow indices were derived from the curve with the largest sum of FEV_1 and FVC. During the measurement, the investigator inspected the volume-time and flow-volume tracings and excluded unacceptable measurements.

Multiple-breath nitrogen washout

MBNW (Exhalyzer D, Ecomedics, Duernten, Switzerland) was performed according to international guidelines,[14] by analyzing the washout pattern of endogenous nitrogen during tidal breathing of 100% oxygen. During the measurement, the investigator monitored the tracings of tidal

volume and marker gas concentrations, and rejected measurements with signs of inert gas leaks. A minimum of 2 successful recordings were required, and mean values were used in the analyses.

Extended exhaled nitric oxide

FE_{NO} was measured with a chemiluminescence analyzer (CLD 88, Ecomedics, Duernten, Switzerland) at four different flow rates: 30, 50, 100, and 200 ml/s, with ≥2 successful measurements required for each flow rate. FE_{NO} was determined at 50 ml/s ($FE_{NO}50$) according to international recommendations.[21] Two-compartment linear model[22] with three flow rates: 100, 200, and either 30 or 50 ml/s for achieving the best goodness of fit (r^2), was used to calculate J_{NO} and C_{ALV}.

Exercise-induced bronchoconstriction

The symptomatic children performed an outdoor free-running test with either IOS or spirometry as described earlier.[23] EIB was defined as an increase of ≥35% in R5[23] or a decrease of ≥15% in FEV_1[24] at 1, 5, or 10 minutes after the exercise.

Eosinophilia and atopy

Eosinophilia was defined as peripheral blood eosinophil count of $\geq 0.4*10^9$/L and eosinophils composing ≥4% of leukocytes. Atopy was defined as serum-specific IgE level of ≥0.35 kU/l or skin prick test wheal diameter of ≥3 mm to ≥1 inhaled aeroallergens: birch, timothy grass, meadow fescue, mugwort, *Cladosporium herbarum*, cat, dog, horse, cow, and *Dermatophagoides pteronyssinus*. Histamine dihydrochloride (10 mg/ml, ALK) served as a positive and physiologic saline as a negative control in the skin prick tests.

Statistical analyses

Power calculations were performed prior to the study for 80% power and 0.05 type I error, with the assumption that the mean AX (standard deviation 0.87 kPa/L)[25] in symptomatic children would differ 0.5 kPa/L from healthy controls, and a 20% margin for possible unsuccessful measurements was used. Accordingly, 58 symptomatic children were recruited.

Due to the lack of applicable reference values that would encompass the age range and nationality of the study children, the data were analyzed with height- and sex-adjusted regression analyses. Normality of the variables was evaluated with Kolmogorov-Smirnov or Shapiro-Wilk test. Comparisons between groups were performed with Mann-Whitney U test or with logistic regression analyses that were adjusted for relevant baseline factors. Receiver operating characteristics (ROC) curves were adjusted for height and sex by plotting the probability of the height- and sex-adjusted logistic regression model. Associations between the proposed small airway indices (R5-20, X5, AX, FEF_{25-75}, LCI, Scond, Sacin, and C_{ALV}) were evaluated using height- and sex-adjusted linear regression analysis. Two-tailed tests with a significance level of 0.05 were used. The data was analyzed with IBM SPSS Statistics Version 23.

Ethics

The study was approved by Helsinki University Hospital (ID number: IAS16 ASA04 0116) and Helsinki University Hospital Research Ethics Committee (approval number: 390/13/03/03/2015). Parents of all participating children provided a written consent, and depending on literacy, the children contributed an oral or written consent.

RESULTS

Baseline characteristics of the healthy and symptomatic children are presented in Table 2. The healthy and symptomatic groups significantly differed only regarding parental smoking. Of the symptomatic children, 14 (24%) used continuous and 8 (14%) used intermittent asthma-control medication which were ceased ≥4 weeks before the diagnostic lung function measurements.

Technical success and reproducibility

All study children performed successful IOS and spirometry measurements. The coherence at 5 Hz was ≥0.80 in 64 (83%) IOS measurements, and coherence at 20 Hz was ≥0.80 in all measurements. Successful $FE_{NO}50$ measurements were obtained from all except for one child. FE_{NO} measurements at all flow rates were successfully performed by 19 (100%) healthy controls and 46 (79%) symptomatic children, with a median r^2 of 0.82 (interquartile range (IQR) 0.73-0.91). Of the healthy controls, 18 (95%) children and 55 (95%) symptomatic children produced successful MBNW measurements with a median coefficient of variation of 5% (IQR 2-10%) for LCI. Successful Scond measurements were achieved in 13 (68%) healthy and 40 (69%) symptomatic children, and successful Sacin measurements in 14 (74%) healthy and 35 (60%) symptomatic children. Children with unsuccessful Scond measurements were significantly younger (p=0.014) than those with successful measurements but no other differences in baseline factors or other lung function indices were observed in children with unsuccessful Scond or Sacin measurements compared to those with successful measurements.

Differences between patients and controls

Baseline lung function of the study children are presented in Table 3. All IOS indices as well as FEV_1/FVC, FEF_{25-75}, and C_{ALV} differed between children with recurrent wheezing and healthy controls. Regarding children with persistent cough, only IOS indices R5 and R20, and spirometry indices FEV_1 and FEF_{25-75} differed from healthy controls. $FE_{NO}50$, J_{NO}, and MBNW indices showed

no difference between the symptomatic children and healthy controls. None of the lung function indices differed between symptomatic children with or without parental smoking (p>0.05). However, separate logistic regression analyses were applied to adjust the parameters for atopic status or parental smoking. After adjusting for atopic status, all associations except for the difference observed in C_{ALV} (p=0.069) between children with recurrent wheezing and healthy controls remained significant. After adjusting for parental smoking, the differences in FEV_1 (p=0.107) and FEF_{25-75} (p=0.088) between children with persistent cough and healthy controls did not remain significant.

Figure 1 presents the height- and sex-adjusted ROC curves illustrating the discriminatory properties of IOS, spirometry, and C_{ALV} between children with recurrent wheezing and healthy controls. All the investigated parameters had an area under the curve (AUC) of >0.70, with R5 and AX providing the best discriminatory power (AUC of >0.80). The unadjusted AUCs for the lung function indices were as follows: 0.857 (95% confidence interval (CI) 0.748-0.965, p<0.001) for R5, 0.705 (95% CI 0.563-0.846, p=0.011) for R5-20, 0.777 (95% CI 0.653-0.901, p=0.001) for X5, 0.791 (95% CI 0.667-0.916, p<0.001) for AX, 0.664 (95% CI 0.523-0.806, p=0.043) for FEV_1/FVC, 0.747 (95% CI 0.614-0.881, p=0.002) for FEF_{25-75}, and 0.686 (95% CI 0.536-0.836, p=0.028) for C_{ALV}.

Associations with asthma control and exercise-induced bronchoconstriction

C-ACT was acquired from 51 symptomatic children with a median score of 24 (range 12-27). A C-ACT score of ≤19 was reported by 11 (31%) of the 36 children with recurrent wheezing and none of the 15 children with persistent cough from whom a filled C-ACT questionnaire was received. None of the lung function parameters were associated with the C-ACT score.

Of the symptomatic children, 23 (40%) reported using SABA within the past month, which was associated with increased $FE_{NO}50$ (p=0.007) and J_{NO} (p=0.027) but not with the other indices of interest. The differences in $FE_{NO}50$ but not in J_{NO} remained significant in the height- and sex-

adjusted logistic regression analyses (p=0.017 and p=0.056, respectively). Sixteen (28%) children required SABA ≤1 time/week, and 7 (12%) ≥2 times/week. Both $FE_{NO}50$ and J_{NO} correlated positively with the increasing use of SABA (r_s=0.400, p=0.004, and r_s=0.377, p=0.017, respectively).

Among the symptomatic children, asthma exacerbations within the past year were reported as follows: no exacerbations in 13 (22%), 1-3 exacerbations in 17 (29%), 4-12 exacerbations in 20 (35%), and >12 exacerbations in 8 (14%) children. Scond, $FE_{NO}50$, J_{NO}, and C_{ALV} correlated positively with the increasing number of exacerbations (r_s=0.340, p=0.032; r_s=0.457, p<0.001; r_s=0.493, p<0.001; and r_s=0.337, p=0.022, respectively). Frequent (≥4 per year) exacerbations were associated with increased Scond, $FE_{NO}50$ (p=0.003), J_{NO}, and C_{ALV} in the symptomatic children (Figure 2). These associations remained significant after adjusting for height, sex, and the use of asthma-control medications (p<0.05).

Of the 55 (95%) symptomatic children who performed the exercise challenge test, 20 (36%) demonstrated significant EIB. LCI, $FE_{NO}50$ (p=0.007), J_{NO}, and C_{ALV} were associated with EIB in the symptomatic children (Figure 3). All associations remained significant in the height- and sex-adjusted logistic regression analysis (p<0.05).

Associations between proposed small airway indices

Significant associations were observed between all the IOS indices (R5-20, X5, and AX) and FEF_{25-75} (p≤0.001 in each comparison), as well as between Scond and C_{ALV} (p=0.035) but not regarding other investigated indices.

DISCUSSION

Despite the increasing clinical and research interest towards small airways, their role in pediatric asthma is poorly understood. We found subtle but significant differences between 5-10-year-old children with mild-to-moderate asthmatic symptoms and healthy controls regarding IOS, spirometry, and FE_{NO} indices related to small airway function, which suggests that small airway dysfunction is present even in mild asthma at this age group. Most of these differences were observed only in children with recurrent wheezing but not in those with persistent cough without wheezing episodes. None of the MBNW indices showed differences in either of the symptomatic subgroups when compared to healthy controls. Among the symptomatic children, Scond and C_{ALV} were associated with asthma exacerbations within the past year, and LCI and C_{ALV} were associated with EIB. However, none of the investigated parameters was associated with C-ACT, and only bronchial NO measures were related to SABA use within the past month.

In children, the postulated small airway IOS indices have been found superior to spirometry in distinguishing asthmatic symptoms and disease control.[3,5,6] Results so far have been ambiguous, however.[26] We found differences in R5-20, X5, and AX, which are proposed to reflect small airway properties, between children with recurrent wheezing and healthy controls, suggesting that these indices might be sensitive in detecting early disease changes of the peripheral airways. Contrary to R5 and R20, however, R5-20, X5, and AX were not associated with persistent cough. Although persistent troublesome cough is associated with later asthma,[27] the symptom can result from a wide variety of causes,[28] and is thus less specific for airway obstruction than recurrent wheezing episodes.[29] Therefore, it is possible that this patient subgroup included children with symptoms resulting from other etiological causes than asthma, blunting the discriminatory power of the investigated indices. Another possible explanation is that this subgroup of children with very mild symptoms presented with too subtle or non-existent changes in the peripheral airway region, and thus remained undetected with the investigated methods.

Based on previous studies, FEF_{25-75} appears superior to FEV_1 in detecting different clinical features of pediatric asthma, such as symptoms and disease control.[30,31] Accordingly, in our study FEF_{25-75} was the most sensitive spirometric parameter for distinguishing between healthy and symptomatic children, and interestingly, the only proposed small airway index that differed from healthy controls in both of the symptomatic subgroups. However, FEF_{25-75} is not specific to small airway obstruction,[13] and therefore, does not confirm the presence of small airway impairment in these subjects.

Increased ventilation inhomogeneity especially in the conducting airways has been reported in asthmatic subjects with well-controlled and mild symptoms when compared to healthy controls.[32-34] These changes have predominantly been subtle and within normal ranges, however.[33,35] Other studies have found differences restricted to severe subgroups.[4] We detected no differences in MBNW indices between healthy and symptomatic children. Regarding Sacin and Scond, strict technical requirements reduced the number of successful measurements, decreasing the power in the statistical analyses, however. In accordance with previous reports,[34,36] we found a weak association between LCI and EIB. In addition, we found an association between Scond and asthma exacerbations within the past year. Therefore, MBNW might offer information on the disease activity, which is supported by the concurrently observed increases in the FE_{NO} indices, especially C_{ALV}. In line with this, multiple-trigger wheezers have been shown to present with conducting airway inhomogeneity.[37] However, the clinical significance of MBNW in pediatric asthma warrants further investigation.

Increased C_{ALV} has predominantly been associated with severe or uncontrolled asthma,[7] and has been observed as less affected in mild disease presentations.[7,9,15,34,38] Accordingly, we found that of the investigated indices, C_{ALV} had the strongest associations with asthma exacerbations and EIB, indicating that the parameter might be useful in reflecting disease control. Also, in line with the proposed small airway IOS indices, C_{ALV} differed from healthy controls only in children with

recurrent wheezing but not in subjects with isolated cough. FE_{NO} measures are, however, influenced by several confounding factors,[39] limiting their diagnostic potential. We also found that after adjusting for atopic status, the difference in C_{ALV} between healthy and wheezing children did not remain significant.

Studies on the association between small airway dysfunction and asthma control questionnaires are conflicting and mainly restricted to adults.[4,26,40] We found no association between the investigated lung function indices and C-ACT scores. However, only 22% of the study subjects reported an uncontrolled disease on the C-ACT, which complicates segregation based on the questionnaire. We did, however, observe a significant association between asthma exacerbations and Scond and C_{ALV}, supporting that peripheral airway dysfunction and inflammation might be associated with an uncontrolled disease.[6,7] We also detected an association between EIB and LCI as well as C_{ALV}, supporting previous reports that small airway dysfunction might be related to airway hyperresponsiveness.[31,34,36]

Functional features related to small airway dysfunction are peripheral ventilation heterogeneity, air trapping, and premature airway closure. All available techniques measure these changes indirectly, and their potential to assess small airways relies on theoretical models which do not elucidate the specific lung region or the physiologic determinants they reflect. Studies on the associations between different methods are scarce and mainly restricted to older children and adults. In accordance with our findings, the postulated small airway IOS indices have been found to correlate with mid-expiratory flow spirometric parameters,[41] whereas C_{ALV} seems unrelated to IOS and spirometry.[40] However, C_{ALV} has been found to correlate with Scond and Sacin in asthmatic children.[34] We found an association between C_{ALV} and Scond but not with Sacin. Also IOS and MBNW indices were unrelated, which is in accordance with previous reports in adults that have found weak or nonexistent associations between IOS and MBW at baseline.[42] Although temporal variability of airway function is characteristic for asthma, we postulated that 1-14 days in-between

the measurements would not have a significant effect on lung function, as no exacerbations or interventions occurred between the measurements.[43,44]

The present study has some limitations regarding the sample size and characteristics. Firstly, as power calculations were performed based on AX, it is possible that the study was underpowered for some of the other parameters, especially Sacin and Scond, which did not show difference between healthy and symptomatic children. Secondly, our patient population consisted of children with mild-to-moderate symptoms in the early disease stage. This setting aimed to minimize the confounding effects of asthma-control medication and a more generalized airway obstruction. It is, however, possible that not all of the symptomatic children present with chronic asthma, especially those with isolated cough. Due to challenges in the differential diagnostics of persistent cough,[28] the discriminatory analyses were performed for both symptomatic subgroups separately, and the ROC analyses only included children with recurrent wheezing. Thirdly, the control group was relatively small and could not be completely matched with symptomatic children with regard to age and gender. Therefore, the statistical analyses were adjusted for height and gender. The lung function measurements of 19 healthy controls did, however, result in a power that was sufficient to detect statistically significant differences in the postulated small airway indices of IOS and spirometry, as well as C_{ALV} when compared to children with recurrent wheezing.

In conclusion, we found subtle but significant differences in the proposed small airway IOS, spirometry, and FE_{NO} indices between children with recurrent wheezing and healthy controls, suggesting that early disease changes in the distal airways might be present in young children with mild asthmatic symptoms, mainly restricted to recurrent wheezing. In addition, ventilation inhomogeneity indices and alveolar inflammation were associated with asthma exacerbations and with EIB. The presence of changes in the proposed small airway indices which were not detected with FEV_1 or FEV_1/FVC highlights the additional value of these measures when assessing children with asthmatic symptoms. Therefore, they might be used as additional measures in the follow-up of

children who present with small airway abnormalities. Further studies are needed to clarify, whether children who present with abnormalities in the proposed small airway indices benefit from treatment with novel therapies targeting the small airways, and which of the available measures are optimal for identifying and following these patients. Of the compared methods, IOS provided the best discriminatory properties between healthy and symptomatic children, whereas C_{ALV} had the strongest associations with asthma exacerbations and EIB. The lack of associations between most of the proposed small airway indices suggests that their physiological determinants may be different.

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FIGURE LEGENDS

Figure 1. Height- and sex-adjusted receiver operating characteristic curves for the discriminative properties of A) respiratory resistance at 5 Hz (R5), difference between respiratory resistance at 5 and 20 Hz (R5-20), respiratory reactance at 5 Hz (X5), and area under the reactance curve (AX), B) forced expiratory volume in 1 second to forced vital capacity (FEV1/FVC) and forced expiratory flow at 25%-75% of the FVC (FEF25-75), and C) alveolar nitric oxide concentration (CALV) between children with recurrent wheezing and healthy controls. AUC, area under the curve; CI, confidence interval.

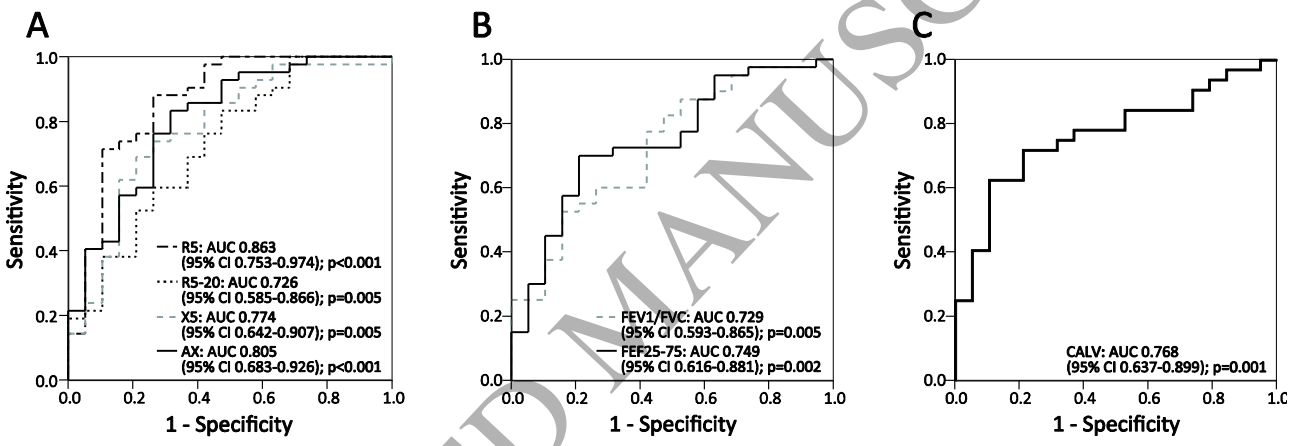


Figure 2. Association between asthma exacerbations within the past year and A) conducting airway ventilation inhomogeneity (Scond), B) bronchial nitric oxide flux (JNO), and C) alveolar nitric oxide concentration (CALV) in the symptomatic children. Statistical analyses were performed using Mann-Whitney U test.

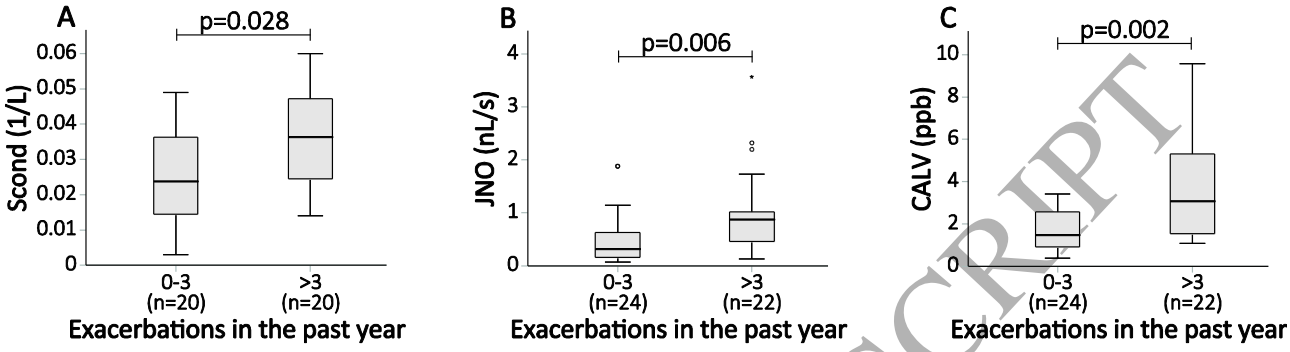
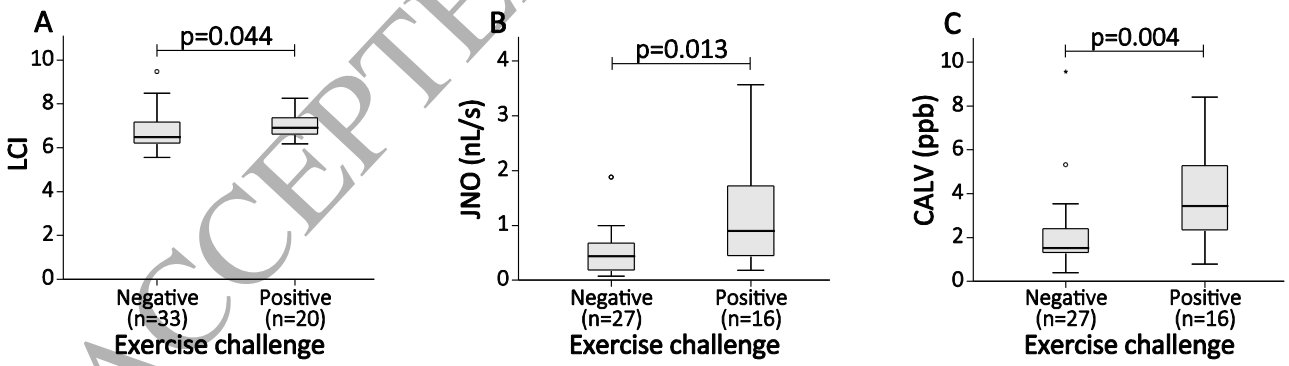


Figure 3. Association between exercise challenge test and A) lung clearance index (LCI), B) bronchial nitric oxide flux (JNO), and C) alveolar nitric oxide concentration (CALV) in the symptomatic children. Statistical analyses were performed using Mann-Whitney U test.



TABLES

Table 1. The postulated small airway indices and their hypothetical physiological determinants.

	Unit	Physiological determinant
IOS		
R5-20	kPa/L/s	Resistive properties of the small airways.
X5	kPa/L/s	Elastic properties of the peripheral lung region.
AX	kPa/L	Integrative measure of the elastic properties of the peripheral lung region.
Spirometry		
FEF_{25-75}	L/s	Airflow limitation in the small airways.
FE_{NO}		
C_{ALV}	ppb	Eosinophilic inflammation in the close to or at the alveolar region.
MBNW		
LCI	–	Total ventilation heterogeneity.
Scond	1/L	Ventilation heterogeneity secondary to small conductive airway dysfunction.
Sacin	1/L	Ventilation heterogeneity secondary to dysfunction in the alveolar region.

Abbreviations: AX, area under the reactance curve; C_{ALV}, alveolar nitric oxide concentration; FEF_{25-75}, forced expiratory flow between 25% and 75% of the forced vital capacity; FE_{NO}, fractional exhaled nitric oxide; IOS, impulse oscillometry; LCI, lung clearance index; MBNW, multiple-breath nitrogen washout test; R5-20, difference between respiratory resistance at 5 and 20 Hz; Sacin, ventilation inhomogeneity in the acinar structures; Scond, ventilation inhomogeneity in the conducting airways; X5, respiratory reactance at 5 Hz.

Table 2. Baseline characteristics of the study children.

	Healthy (n=19)	Recurrent wheezing (n=42)	Persistent cough (n=16)	All symptomatic (n=58)
Boys (%)	7 (37%)	26 (62%)	10 (63%)	36 (62%)
Age (years)	7.4 (6.2; 9.3)	6.9 (6.1; 7.8)	7.0 (6.4; 8.4)	7.0 (6.2; 8.2)
Height (cm)	130 (122; 139)	124 (118; 132)	127 (118; 132)	125 (118; 132)
Weight (kg)	27 (22; 30)	25 (21; 33)	25 (21; 31)	25 (21; 32)
Low birth weight[a]	0	0	2 (13%)	2 (3%)
Prematurity[b]	0	1 (2%)	2 (13%)	3 (5%)
Eosinophilia[c]	6 (32%)	24 (57%)	8 (50%)	32 (55%)
Atopy[d]	9 (47%)	30 (71%)	6 (38%)	36 (62%)
Parental Asthma	6 (32%)	14 (33%)	3 (19%)	17 (29%)
Parental Smoking	0	14 (33%)*	7 (44%)*	21 (36%)*

Data presented as median (interquartile range) or n (n%).

[a]Birth weight of <2500 grams.

[b]Gestational age of <36 weeks.

[c]Peripheral blood eosinophils of $\geq 0.4*10^9$/L and ≥4%.

[d]Serum-specific IgE level of ≥0.35 kU/l or skin prick test wheal diameter of ≥3 mm for birch, timothy grass, meadow fescue, mugwort, *Cladosporium herbarum*, cat, dog, horse, cow, or *Dermatophagoides pteronyssinus*.

*P<0.05 compared to the healthy control group using Chi-squared test.

Table 3. Baseline lung function of the study children.

	Healthy (n=19)	Recurrent wheezing (n=42)	p*	Persistent cough (n=16)	p*
IOS					
R5 (kPa/L/s)	0.58 (0.50; 0.69)	0.79 (0.73; 0.91)	**<0.001**	0.79 (0.59; 1.01)	**0.016**
R20 (kPa/L/s)	0.45 (0.41; 0.53)	0.59 (0.53; 0.64)	**<0.001**	0.58 (0.51; 0.71)	**0.006**
R5-20 (kPa/L/s)	0.14 (0.08; 0.21)	0.21 (0.15; 0.29)	**0.047**	0.15 (0.08; 0.32)	0.260
X5 (kPa/L/s)	-0.18 (-0.21; -0.16)	-0.25 (-0.31; -0.20)	**0.013**	-0.23 (-0.29; -0.19)	0.073
AX (kPa/L)	1.01 (0.49; 1.87)	1.89 (1.48; 2.67)	**0.004**	1.95 (0.85; 2.95)	0.061
Spirometry					
FEV_1 (L)	1.67 (1.43; 2.00)	1.54 (1.27; 1.71)	0.160	1.45 (1.20; 1.58)	**0.031**
FVC (L)	1.88 (1.66; 2.17)	1.71 (1.40; 2.08)	0.913	1.64 (1.37; 1.92)	0.170
FEV_1/FVC	0.89 (0.85; 0.92)	0.85 (0.81; 0.91)	**0.034**	0.86 (0.81; 0.90)	0.216
FEF_{25-75} (L/s)	2.01 (1.63; 2.55)	1.59 (1.39; 1.89)	**0.024**	1.65 (1.33; 1.91)	**0.045**
FE_{NO}					
FE_{NO}50 (ppb)	8.50 (4.00; 13.80)	12.90 (7.40; 23.33)	0.073	9.30 (4.00; 13.90)	0.596
J_{NO} (nL/s)	0.35 (0.17; 0.56)	0.67 (0.32; 0.99)	0.075	0.22 (0.13; 0.52)	0.979
C_{ALV} (ppb)	1.35 (1.16; 2.83)	2.36 (1.54; 4.56)	**0.046**	1.44 (0.64; 2.70)	0.745
MBNW					
FRC (L)	1.44 (1.09; 1.57)	1.13 (1.00; 1.43)	0.514	1.18 (1.03; 1.59)	0.612
LCI	6.81 (6.41; 7.33)	6.87 (6.52; 7.47)	0.455	6.43 (6.08; 6.55)	0.459
Scond (1/L)	0.022 (0.019; 0.037)	0.035 (0.021; 0.042)	0.494	0.026 (0.020; 0.034)	0.969
Sacin (1/L)	0.095 (0.043; 0.158)	0.147 (0.049; 0.192)	0.803	0.114 (0.042; 0.192)	0.777

Data presented as median (interquartile range).

*P-value compared to healthy control group using height-and sex-adjusted logistic regression model.

Abbreviations: AX, area under the reactance curve; C_{ALV}, alveolar nitric oxide concentration; FEF_{25-75}, forced expiratory flow between 25% and 75% of the forced vital capacity; FE_{NO},

fractional exhaled nitric oxide; $FE_{NO}50$, FE_{NO} at 50 ml/s; FEV_1, forced expiratory volume in 1 second; FRC, functional residual capacity; FVC, forced vital capacity; IOS, impulse oscillometry; J_{NO}, bronchial nitric oxide flux; LCI, lung clearance index; MBNW, multiple-breath nitrogen washout test; R5 and R20, respiratory resistance at 5 and 20 Hz, respectively; R5-20, difference between respiratory resistance at 5 and 20 Hz; Sacin, ventilation inhomogeneity in the acinar structures; Scond, ventilation inhomogeneity in the conducting airways; X5, respiratory reactance at 5 Hz.